VERIFICATION OF TRANSLATION

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hereby declare that I am the translator of the document attached and certify that the following is true translation to the best of my knowledge and belief.

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[Document Name] Specification

[Title of the Invention] FREEZE-DRIED INTERFERON COMPOSITION FOR TRANSPULMONARY ADMINISTRATION

[Claims]

[Claim 1] A freeze-dried composition for transpulmonary administration having the following properties (i) to (iv):

(i) containing at least one hydrophobic stabilizer selected from the group consisting of hydrophobic amino acids, dipeptides of hydrophobic amino acids, tripeptides of hydrophobic amino acids and derivatives of hydrophobic amino acids and salts thereof; at least one hydrophilic stabilizer selected from the group consisting of hydrophilic amino acids, dipeptides of hydrophilic amino acids, tripeptides of hydrophilic amino acids, derivatives of hydrophilic amino acids and salts thereof; and interferon-γ

(ii) a non-powder cake-like form;

(iii) a disintegration index of 0.015 or more; and

(iv) becoming fine particles having a mean particle diameter of 10 microns or less or a fine particle fraction of 10% or more upon receipt of an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec.

[Claim 2] The freeze-dried composition

according to Claim 1, wherein the hydrophilic stabilizer is at least one selected from the group consisting of basic amino acids, neutral hydroxy amino acids, dipeptides of these amino acids, tripeptides of these amino acids, derivatives of these amino acids and salts thereof.

[Claim 3] The freeze-dried composition according to Claim 1 or 2, wherein the hydrophilic stabilizer is at least one selected from the group consisting of basic amino acids, dipeptides of basic amino acids, tripeptides of basic amino acids, derivatives of basic amino acids and salts thereof.

[Claim 4] The freeze-dried composition according to Claim 1 or 2, wherein the hydrophilic stabilizer is at least one selected from the group consisting of neutral hydroxy amino acids, dipeptides of neutral hydroxy amino acids, tripeptides of neutral hydroxy amino acids, derivatives of neutral hydroxy amino acids and salts thereof.

[Claim 5] The freeze-dried composition according to Claim 1 or 2, wherein the hydrophilic stabilizer is at least one selected from the group consisting of arginine, lysine, histidine, threonine, dipeptide of these amino acids, tripeptides of these amino acids, derivatives of these amino acids and salts thereof.

[Detailed Description of the Invention]

[0001]

[Technical Field to Which the Invention Pertains]

The present invention relates to a freeze-dried composition containing interferon-γ for transpulmonary administration. More specifically, the present invention relates to a freeze-dried interferon-γ composition which can stably maintain interferon-γ and can be made into fine particle powder suitable for transpulmonary administration (hereinafter, referred to as dry powdered interferon-γ preparation for transpulmonary administration) at the time of use.

[0002]

[Prior Art]

In general, with regard to transpulmonary administration, it is known that the active ingredient contained in a medicine can be delivered into the lungs efficiently by making the mean particle diameter of the active ingredient be 10 microns or less, preferably 5 microns or less. The current situation with conventional inhalations for transpulmonary administration is thus that, to make the medicine have a particle diameter suitable for transpulmonary administration in advance, fine particles are prepared by a spray drying method, a

jet milling method or the like, and possibly further processing is carried out, and then the fine particles are provided filled into a dry powder inhaler (for example, Patent Documents 1, 2, etc.).

[0003]

Moreover, conventional methods for preparing dry powdered inhalation for transpulmonary administration require an operation in which the fine powder prepared is collected from the spray drying apparatus or jet milling apparatus and is subdivided and filled into vessels. It is thus inevitable that, accompanying this operation, problems will arise such as the yield of the preparation decreasing due to collection or filling loss and the cost rising correspondingly, and the preparation being contaminated with impurities. Moreover, in general it is difficult to subdivide and fill the powder in small amounts with good accuracy. If the spray drying method or the freeze drying-jet milling method, for which such subdividing and filling of small amounts in powder form is essential, is used, then it is thus necessary to establish a method of filling with small amounts and good accuracy of powder. In actual fact, details of a system, apparatus and method for filing with a fine powder are disclosed in U.S.Patent NO. 5,826,633.

[0004]

Interferons are well known as active ingredients capable of being used for transpulmonary administration, and which have biological properties such as antiviral properties, immune modulating properties or cell proliferation suppressing properties. Interferons are proteins and thus inherently prone to lose activity due to heat, pH and the like. In particular, interferon-γ among various types of interferon has disadvantages such that the activity is easily lost and stability is poor. Therefore, a dry powdered inhalation for transpulmonary administration containing interferon-γ as an active ingredient is disadvantaged in that the activity of interferon-γ is reduced during formulation or with time, in addition to the problems of the conventional dry powdered inhalations for transpulmonary administration.

[0005]

[Patent Document 1]

International Publication No. WO 95/31479

[0006]

[Patent Document 2]

International Publication No. WO91/16038

[0007]

[Problem to Be Solved by the Invention]

It is an object of the present invention to solve the various problems of the above-mentioned conventional

powdered inhalations for transpulmonary administration. Specifically, it is an object of the present invention to provide a freeze-dried composition for transpulmonary administration which can stably maintain interferon-γ and can be made into fine particles in the vessel at the time of usage.

[0008]

[Means for Solving the Problem]

The present inventors carried out assiduous studies to attain the above object, and as a result discovered that interferon-γ having the following properties (i) to (iv) can be made into fine particles by a relatively low air impact while still housed in the vessel and interferon-γ in the composition is endowed with excellent stability: (i) at least one hydrophobic stabilizer selected from the group consisting of hydrophobic amino acids, dipeptides of hydrophobic amino acids, tripeptides of hydrophobic amino acids, derivatives of hydrophobic amino acids and salts thereof; at least one hydrophilic stabilizer selected from the group consisting of hydrophilic amino acids, dipeptides of hydrophilic amino acids, tripeptides of hydrophilic amino acids and derivatives of hydrophilic amino acids and salts thereof; and interferon-γ; (ii) having a non-powder cake-like form; (iii) having a disintegration

index of at least 0.015; and (iv) upon receiving an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec becoming fine particles having a mean particle diameter of no more than 10 microns or a fine particle fraction of at least 10%. The present invention was developed based on this knowledge.

[0009]

The present invention includes the following freeze-dried compositions for transpulmonary administration.

Item 1. A freeze-dried composition for transpulmonary administration having the following properties (i) to (iv):

(i) containing at least one hydrophobic stabilizer selected from the group consisting of hydrophobic amino acids, dipeptides of hydrophobic amino acids, tripeptides of hydrophobic amino acids, derivatives of hydrophobic amino acids and salts thereof; at least one hydrophilic stabilizer selected from the group consisting of hydrophilic amino acids, dipeptides of hydrophilic amino acids, tripeptides of hydrophilic amino acids, derivatives of hydrophilic amino acids and salts thereof; and interferon-γ;

(ii) a non-powder cake-like form;

(iii) a disintegration index of 0.015 or more; and

(iv) becoming fine particles having a mean particle diameter of 10 microns or less or a fine particle fraction of 10% or more upon receiving an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec.

Item 2. The freeze-dried composition according to Item 1, wherein the hydrophilic stabilizer is at least one selected from the group consisting of basic amino acids, neutral hydroxy amino acids, dipeptides of these amino acids, tripeptides of these amino acids, derivatives of these amino acids and salts thereof.

Item 3. The freeze-dried composition according to Item 1 or 2, wherein the hydrophilic stabilizer is at least one selected from the group consisting of basic amino acids, dipeptides of basic amino acids, tripeptides of basic amino acids, derivatives of basic amino acids and salts thereof.

Item 4. The freeze-dried composition according to Item 1 or 2, wherein the hydrophilic stabilizer is at least one selected from the group consisting of neutral hydroxy amino acids, dipeptides of neutral hydroxy amino acids, tripeptides of neutral hydroxy amino acids, derivatives of neutral hydroxy amino acids and salts thereof.

Item 5. The freeze-dried composition according to Item 1 or 2, wherein the hydrophilic stabilizer is at

least one selected from the group consisting of arginine, lysine, histidine, threonine, dipeptides of these amino acids, tripeptides of these amino acids, derivatives of these amino acids and salts thereof.

Item 6 The freeze-dried composition according to Item 1, 2 or 5, wherein the hydrophilic stabilizer is at least one selected from the group consisting of arginine, lysine, histidine, threonine, and salts thereof.

Item 7 The freeze-dried composition according to Item 1, 2, 5 or 6, wherein the hydrophilic stabilizer is at least one selected from the group consisting of arginine, and salts thereof.

[0010]

Hereinafter, in this specification, the term "fine particles" includes pulverized powder (particle powder).

[0011]

[Mode for Carrying Out the Invention]

The freeze-dried composition of the present invention is a composition containing interferon-γ, hydrophobic stabilizer and hydrophilic stabilizer.

[0012]

IFN-γ employed in the present invention is not limited in origin. Such IFN-γ includes natural IFN-γ produced using cell culture technology or recombinant IFN-γ produced using DNA recombinant technology, such as

IFN-γ 1a, IFN-γ 1b and IFN-γ disclosed in Japanese Published Patent Nos. 1994-173196, 1997-19295, etc.

[0013]

In the present invention, hydrophobic stabilizers include hydrophobic amino acids, dipeptides of hydrophobic amino acids, tripeptides of hydrophobic amino acids, derivatives of hydrophobic amino acids and salts thereof.

[0014]

In the present invention, hydrophobic amino acids include protein-forming amino acids such as valine, leucine, isoleucine, phenylalanine and the like. Dipeptides of hydrophobic amino acids are dipeptides having at least one hydrophobic amino acid and include leucyl-valine, isoleucyl-valine, isoleucyl-leucine, leucyl-glycine and the like. Tripeptides of hydrophobic amino acids are tripeptides having at least one hydrophobic amino acid and include isoleucyl-leucyl-valine, leucyl-glycyl-glycine, and the like. Derivatives of hydrophobic amino acids include amides of hydrophobic amino acids such as L-leucine amido hydrochloride, L-isoleucyl-β-naphthylamido hydrobromide, L-valine-β-naphthyl amide, and the like. Salts include those with an alkali metal such as sodium or potassium; with an alkaline earth metal such as calcium

or magnesium; and addition salts with an inorganic acid such as phosphoric acid, hydrochloric acid or hydrobromic acid; or addition salts with an organic acid such as sulfonic acid.

[0015]

Hydrophobic stabilizers include preferably valine, leucine, isoleucine, phenylalanine, and salts thereof.

[0016]

Such hydrophobic stabilizers can be used alone or in combination of two or more.

[0017]

In the present invention, the hydrophilic stabilizers include hydrophilic amino acids, dipeptides of hydrophilic amino acids, tripeptides of hydrophilic amino acids, derivatives of hydrophilic amino acids, and salts thereof.

[0018]

The hydrophilic amino acids employed in the present invention may be any amino acids insofar as they have a hydrophilic side chain, whether or not the amino acids are protein forming amino acids. Specific examples of the hydrophilic amino acids include basic amino acids such as arginine, lysine, histidine, etc.; neutral hydroxy amino acids such as serine, threonine, etc.;

acidic amino acids such as aspartic acid, glutamic acid, etc.; amide amino acids such as asparagine, glutamine, etc.; and other amino acids such as glycine, alanine, cysteine, tyrosine and the like. Basic amino acids are amino acids having basic side chains. Neutral hydroxy amino acids have hydroxyl groups on the side chains. Dipeptides of hydrophilic amino acids have two of the same or different the hydrophilic amino acids. Tripeptides of hydrophilic amino acids have three of the same or different hydrophilic amino acids. Derivatives of hydrophilic amino acids include amides of the hydrophilic amino acids, etc. Salts include those with an alkali metal such as sodium, potassium, etc.; with an alkaline earth metal such as calcium, magnesium, etc.; and addition salts with an inorganic acid such as phosphoric acid, hydrochloric acid or hydrobromic acid, etc., or with an organic acid such as sulfonic acid. Specific examples include salts of hydrophilic amino acids such as arginine hydrochloride, lysine monohydrochloride, lysine dihydrochloride, histidine hydrochloride, histidine dihydrochloride, etc.

[0019]

The hydrophilic stabilizer preferably include basic amino acids, neutral hydroxy amino acids, dipeptides of these amino acids, tripepdides of these amino acids,

derivatives of these amino acids; basic amino acids, dipeptides of the basic amino acids, tripeptides of the basic amino acids, derivatives of the basic amino acids and salts thereof; neutral hydroxy amino acids, dipeptides of neutral hydroxy amino acids, tripeptides of neutral hydroxy amino acids, derivatives of neutral hydroxy amino acids and salts thereof; arginine, lysine, histidine, threonine, dipeptides of these amino acids, tripeptides of these amino acids, derivatives of these amino acids and salts thereof: arginine, lysine, histidine, threonine and salts thereof; arginine, lysine, histidine and salts thereof: and arginine and salts thereof.

[0020]

These hydrophilic amino stabilizes can be used alone or in combination of two or more.

[0021]

The content of IFN-γ of the freeze-dried composition for transpulmonary administration can be set according to a target disease, expected effects and the like. The proportion of IFN-γ may for example be in the range of 0.01 to 99.8 wt% of the composition, preferably in the range of 0.1 to 95 wt%, and more preferably in the range of 0.1 to 90 wt%.

[0022]

The content of hydrophobic stabilizer of the freeze-dried composition for transpulmonary administration can be set according to the proportion of IFN-γ, the type of the hydrophobic stabilizer to be used, disintegration index of the composition, etc. For example, the proportion of the hydrophobic stabilizer may be in the range of 0.1 to 99.89 wt%, preferably within the range of 1 to 95 wt%, and more preferably 5 to 90 wt%.

[0023]

The proportion of hydrophilic stabilizer of the freeze-dried composition for transpulmonary administration varies according to the content of IFN-γ, the proportion of the hydrophilic stabilizer, and the type of the hydrophilic stabilizer to be used, and thus cannot be determined uniformly, but may be in the range of 0.1 to 99.89 wt%, preferably within the range of 1 to 90 wt%, more preferably 2 to 80 wt%, and more preferably 5 to 70 wt%.

[0024]

The proportion of hydrophilic stabilizer to hydrophobic stabilizer contained in the freeze-dried composition for transpulmonary administration should be 1 to 500 parts by weight of hydrophilic stabilizer per 100 parts by weight of the hydrophobic stabilizer, preferably 2 to 400 parts by weight, more preferably 5

to 300 parts by weight, still more preferably 8 to 250, and in particular preferably 10 to 200 parts by weight.

[0025]

The amount of IFN-γ contained in a unit dose (single dose) of the freeze-dried composition for transpulmonary administration is 10,000 to 50,000,000 IU (International Units), preferably 100,000 to 40,000,000 IU, and more preferably 100,000 to 30,000,000 IU.

[0026]

The amount of hydrophobic stabilizer contained in a single dose of the freeze-dried composition for transpulmonary administration is in the range of 0.01 to 10 mg, preferably 0.1 to 5 mg, and more preferably 0.2 to 0.5 mg.

[0027]

The amount of hydrophilic stabilizer contained in a single dose of the freeze-dried composition for transpulmonary administration is in the range of 0.01 to 10 mg, preferably 0.1 to 5 mg, and more preferably 0.1 to 2.5 mg.

[0028]

As described above, hydrophobic stabilizer and hydrophilic stabilizer are mixed with the freeze-dried composition for transpulmonary administration, and thus, the composition can satisfy the desired disintegration

index (described later), and IFN-γ of the composition can be endowed with excellent stability.

[0029]

In addition to the above ingredients, the freeze-dried composition for transpulmonary administration of the present invention can further include monosaccharides such as glucose; disaccharides such as saccharose, maltose, lactose and trehalose; sugar alcohols such as mannitol; oligosaccharides such as cyclodextrin; polysaccharides such as dextran 40 and pullulan; polyhydric alcohols such as polyethylene glycol; fatty acid sodium salts such as sodium caprate; human serum albumin; inorganic salts; surfactants; buffering agents and so on, as long as the end products satisfy the above-mentioned disintegration index. A wide range of surfactants can be used, regardless of whether they are anionic surfactants, cationic surfactants or nonionic surfactants, provided that they are surfactants that are generally used in medicines. Preferable examples are nonionic surfactants such as sorbitan trioleate and polyoxyethylene sorbitan fatty acid esters (for example Tween type surfactants).

[0030]

The freeze-dried composition for transpulmonary administration of the present invention is a freeze-dried

composition having a non-powder cake-like form. In the present invention, 'non-powder cake-like form freeze-dried composition' means a dry solid obtained by freeze-drying a solution, and is generally called a 'freeze-dried cake'. However, even if cracks appear in the cake, the cake breaks into a plurality of large lumps, or part of the cake breaks into a powder during the freeze-drying process or during subsequent handling, this cake is still included as a non-powder-form freeze-dried composition that is the subject of the present invention, provided the effects of the present invention are not impaired.

[0031]

The freeze-dried composition for transpulmonary administration of the present invention has a disintegration index of at least 0.015. Note that the disintegration index in the present invention is a value characteristic of the freeze-dried composition that can be obtained by measuring following the undermentioned method.

<Disintegration index>

0.2 to 0.5 ml of a mixture containing target components that will constitute the freeze-dried composition is filled into a vessel having a trunk diameter of 18 mm or 23 mm, and freeze-drying is carried

out. Next, 1.0 ml of n-hexane is instilled gently down the wall of the vessel onto the non-powder-form freeze-dried composition obtained. Agitation is carried out for about 10 seconds at 3000 rpm, and then the mixture is put into a UV cell of optical path length 1 mm and optical path width 10 mm, and the turbidity is measured immediately at a measurement wavelength of 500 nm using a spectrophotometer. The turbidity obtained is divided by the total amount (weight) of the components constituting the freeze-dried composition, and the value obtained is defined as the disintegration index.

[0032]

Here, an example of the lower limit of the disintegration index of the freeze-dried composition of the invention can be given as the above-mentioned 0.015, preferably 0.02, more preferably 0.03, yet more preferably 0.04, and still more preferably 0.05. In particular, 0.1 or 0.15 is preferable. Moreover, there is no particular limitation on the upper limit of the disintegration index of the freeze-dried composition of the invention, but an example can be given as 1.5, preferably 1, more preferably 0.9, yet more preferably 0.8, still more preferably 0.7. The freeze-dried composition of the present invention preferably has a disintegration index in a range constituted from a lower

limit and an upper limit selected as appropriate from the above, with the proviso that the disintegration index is at least 0.015. Specific examples of the range of the disintegration index are 0.015 to 1.5, 0.02 to 1.0, 0.03 to 0.9, 0.04 to 0.8, 0.05 to 0.7, 0.1 to 0.7, 0.15 to 1.5, 0.15 to 1.0, and 0.15 to 0.7.

[0033]

The freeze-dried composition of the present invention also has a property of becoming fine particles having a mean particle diameter of 10 microns or less or a fine particle fraction of 10% or more upon receipt of an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec on the basis of properties peculiar to the freeze-dried composition represented by the disintegration index.

[0034]

As used herein, the mean particle diameter of fine particles indicates a mean particle diameter usually adopted in the industry relating to inhalants for transpulmonary administration. Specifically, the mean particle diameter is not a geometric particle diameter, but an aerodynamic mean particle diameter (mass median aerodynamic diameter, MMAD). The aerodynamic mean particle diameter can be measured by a conventional method. For example, the mass median aerodynamic

diameter can be measured using a dry particle size distribution meter fitted with an Aerobreather, which is an artificial lung model (made by Amherst Process Instrument, Inc., USA), a twin impinger (G.W. Hallworth and D.G. Westmoreland: J. Pharm. Pharmacol., 39, 966-972 (1987), U.S.Patent No. 6153224), a multi-stage liquid impinger, a Marple-Miller impactor, an Andersen cascade impactor or the like. Moreover, B. Olsson et al. have reported that delivery of the particles into the lungs increases at the proportion of particles having a mass median aerodynamic diameter of 5μm or less increases (B. Olsson et al. : Respiratory Drug Delivery V, 273-281(1996)). The fine particle fraction, fine particle dose or the like as measured by a twin impinger, a multi-stage liquid impinger, a Marple-Miller impactor, an Andersen cascade impactor or the like acts as a method of estimating the amount that can be delivered into the lungs.

[0035]

A preferable freeze-dried composition for transpulmonary administration is one such that, upon receipt of an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec, the mean particle diameter becomes 10 microns or less and preferably 5 microns or less or a fine particle fraction

of 10% or more, preferably 20% or more, more preferably 25% or more, still more preferably 30% or more, and especially more preferably 35% or more.

[0036]

As described above, the air impact applied to a freeze-dried composition is not limited, as long as it is generated by air having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec. Specific examples of an air impact include an impact generated by an air having a speed of 1 m/sec or more, preferably 2 m/sec or more, more preferably 5 m/sec or more and a still more preferably 10 m/sec or more. Here, there is no limitation on the upper limit of the air speed, but it is generally 300 m/sec, preferably 250 m/sec, more preferably 200 m/sec and yet more preferably 150 m/sec. The air speed is not limited as long as it is arbitrary selected from the range extending from a lower limit to an upper limit; however, the ranges of 1 to 300 m/sec, 1 to 250 m/sec, 2 to 250 m/sec, 5 to 250 m/sec, 5 to 200 m/sec, 10 to 200 m/sec or 10 to 150 m/sec can be given as examples.

[0037]

Examples of the air impact include those generated by air having an air flow rate of generally 17 ml/sec or more, preferably 20 ml/sec or more and more preferably

25 ml/sec or more. There is no limitation on the upper limit of the air flow rate; however, the air flow rate is generally 900 L/min, preferably 15 L/sec, more preferably 5 L/sec and yet more preferably 4 L/sec. Especially, 3 L/sec is very preferable. More specifically, the air flow rate is not limited as long as it is selected from the range extending from a lower limit to an upper limit; however, examples of such a range include 17 ml/sec to 15 L/sec, 20 ml/sec to 10L/sec, 20 ml/sec to 5 L/sec, 20 ml/sec to 4 L/sec, 20 ml/sec to 3 L/sec and 25 ml/sec to 3 L/sec.

[0038]

The freeze-dried composition for transpulmonary administration of the present invention is manufactured by preparing a solution containing IFN-γ, hydrophobic stabilizer and hydrophilic stabilizer, filling the solution of an amount corresponding to a unit dose (single dose) or a plurality of doses into the vessel, and freeze-drying the same. Such a freeze-dried composition for transpulmonary administration can be manufactured by standard freeze-drying methods commonly used in preparing freeze-dried preparations (freeze-dried compositions), such as an injection preparation which is dissolved at the time of usage. In the process of manufacturing the freeze-drying composition of the

present invention, the freeze-dried composition for transpulmonary administration is prepared so that, for example, a single dose of IFN-γ is included in the vessel, whereby the composition as is can be made into fine particles having a particle diameter suitable for transpulmonary administration in the vessel immediately before transpulmonary administration, and then the powdered composition can be inhaled as is (transpulmonary administration) from the vessel.

[0039]

The freeze-dried composition for transpulmonary administration thus obtained can be prepared into fine particles suitable for transpulmonary administration by an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec. A device for inhaling (transpulmonary administration) the powdered composition includes a dry powder inhaler provided with a means capable of applying an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec to the freeze-dried composition in the vessel and a means for discharging the powder-form freeze-dried composition having fine particles. Therefore, the above-described device is combined with the vessel housing the freeze-dried composition for transpulmonary administration containing a single dose of IFN-γ, whereby

the freeze-dried composition which has been provided in a non-powder form into a powdered preparation comprising fine particles having a mean particle diameter of 10 microns or less or a fine particle fraction of 10% or more, which is a preparation suitable for transpulmonary administration, can be prepared by a user himself/herself at the time of use (the time of inhalation), and administer (take) the powdered preparation.

[0040]

The amount of a single dose of the freeze-dried composition for transpulmonary administration of the present invention can be set according to the target disease, expected effects, types of IFN-γ contained, etc. For example, the amount of the single dose may be 0.1 to 20 mg, preferably 0.2 to 15 mg, more preferably 0.3 to 10 mg, still more preferable 0.4 to 8 mg, and in particular preferably 0.5 to 5 mg.

[0041]

The freeze-dried composition having a non-powder cake-like form containing various active ingredients such as proteins, peptides, polypeptides, genes, nucleic acids, low-molecular-weight drugs; and carriers such as amino acids, sugars, etc., if required, can be made into fine particles having a smaller mean particle diameter or a higher proportion of effective particles (fine

particle fraction) upon receipt of an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec when the amount of salts included in the composition is small. Thus, it is preferable to reduce the concentration of salts contained in the solution used for the freeze-drying process, thereby preparing a freeze-dried composition for transpulmonary administration which can be made into fine particles such that the mean particle diameter can be reduced and the effective particles (fine particle fraction) can be increased upon receipt of an air impact. For example, when salts are contained as preservatives or stabilizers in the refined powder of the active ingredients used or solution, the concentration of the salts contained in the solution used for the freeze-drying process can be reduced either by prior desalination of the refined powder of the active ingredients used or solution or by desalinating the solution itself used for the freeze-drying process. The desalinating method is not limited, but includes ultrafiltration, precipitation, ion-exchange, dialysis under reduced pressure, etc.

[0042]

[Examples]

Following is a detailed description of the present invention, citing examples; however, the present

invention is not limited to these examples.

[0043]

In the following examples, the disintegration index of the non-powder-form freeze-dried composition (freeze-dried cake) of the present invention, and the fine particle fraction (%), which is an indicator for evaluating the delivery into the lungs of the dry powdered preparation produced, were calculated in accordance with the following methods.

<Calculation of disintegration index>

1.0 ml of n-hexane is instilled gently down the wall of the vessel into the prepared non-powder-form freeze-dried composition (freeze-dried cake), and agitation is carried out for about 10 seconds at 3,000 rpm using an Automatic Lab-Mixer NS-8 (made by Pasolina). The mixture obtained is put into a UV cell (made by Shimadzu GLC Center) of optical path length 1 mm and optical path width 10 mm, and then the turbidity of the mixture is measured immediately at a measurement wavelength of 500nm using a spectrophotometer (UV-240, made by Shimadzu Corporation). The value obtained by dividing the turbidity obtained by the total formulation amount (the total amount (weight) of the active ingredient and the carrier) is taken as the disintegration index.

[0044]

<Calculation of fine particle fraction>

A vessel filled with the prepared freeze-dried composition is installed into the dry powder inhaler, and using the device a prescribed air impact is applied on the composition, and the fine powdered preparation thus produced is discharged directly into Apparatus A (a twin impinger: made by Copley, UK) as mentioned in the European Pharmacopoeia (Third Edition Supplement 2001, p113-115). After this, the solvents in stage 1 and stage 2 of the apparatus are respectively collected, and the active ingredient contained in each solvent in the stage 1 or stage 2 is assayed using an appropriate method in accordance with the type of active ingredient in the freeze-dried composition, for example a bioassay method or HPLC (see the report of Lucas et al. (Pharm. Res., 15 (4), 562-569 (1998)) and the report of Iida et al. (Yakugaku Zasshi, 119 (10), 752-762 (1999)). The fraction that can be expected to be delivered into the lungs is that in stage 2 (the aerodynamic diameter of particles recovered in this fraction is 6.4 μm or less); the proportion of the active ingredient that reaches stage 2 and is recovered here is generally called the fine particle fraction (the amount that can be expected to reach the lungs), and is taken as a yardstick for

evaluating the suitability as an inhalation for transpulmonary administration.

[0045]

In Examples and Comparative Examples given below, the active ingredients contained in stage 1 and stage 2 were quantitated, and the weight amount of the active ingredient in stage 2 was divided by the total weight amount of the active ingredients jetted out (the total weight amount of the active ingredients contained in stage 1 and stage 2: hereinafter also referred to as "Stage 1 + Stage 2") to calculate fine particles fraction. Moreover, as a rule in the European Pharmacopoeia, when using the twin impinger (made by Copley, UK), it is stipulated that suction is carried out at an air suction flow rate of 60 L/min, i.e. 1 L/sec, and hence in the examples and comparative examples below this was followed.

[0046]

Examples 1 to 4

An interferon-γ (IFN-γ) stock liquid (potency: 1×10^7 IU/ml) was desalinated using an ultrafilter membrane (Ultrafree 15, manufactured by Millipore). 100,000 IU of the desalinated IFN-γ obtained and various carriers having the amount shown in Table 1 below were dissolved into distilled water for an injection such that the volume

was 0.5 ml and the resultant was filled into vessels (trunk diameter 18 mm), and freeze-drying was carried out using a shelf-type freeze-dryer (Lyovac GT-4, made by Leybold) (Examples 1 to 4 and Comparative Examples 1 and 2).

[0047]

The disintegration index of the non-powder-form (cake-like) freeze-dried composition (freeze-dried cake) obtained was calculated.

[0048]

Moreover, to calculate the fine particle fraction (%) of the fine particles for each freeze-dried composition and thus evaluate the efficiency of delivery into the lungs, an air impact arising through an air speed of about 35 m/sec and an air flow rate of about 40 ml/sec was applied to the freeze-dried cake filled into a vessel using the dry powder inhaler, and the resulting powdered fine-particle-form freeze-dried composition was discharged directly into a twin impinger (made by Copley, UK). After this, the solvents in stage 1 and stage 2 were collected, and the IFN-γ in the stage 1 and stage 2 solvents were assayed using a bioassay method. The value obtained by dividing the amount (weight) of IFN-γ obtained in stage 2 by the total amount (weight) of IFN-γ jetted out (stage 1 + stage 2) was then calculated as the fine particle fraction (%).

[0049]

To evaluate stability of IFN-γ of the freeze-dried composition obtained, residual activity of IFN-γ immediately after freeze-drying (hereinafter, referred to as residual activity after freeze-drying) compared to activity (100%) of IFN-γ immediately before freeze-drying, and residual activity of IFN-γ after preserved at 70°C for two weeks (hereinafter, referred to as residual activity after high-temperature preservation) compared to activity (100%) of IFN-γ immediately after freeze-drying was assayed by bioassay method.

[0050]

The disintegration index, fine particle fraction (%), residual activity after freeze-drying (%) and residual activity (%) after high-temperature preservation for each freeze-dried composition (Examples 1 to 4 and Comparative Examples 1 and 2) are shown in Table 1.

[Table 1]

	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Com. Ex. 1	Com.Ex. 2
IFN-γ	100,000IU	100,000IU	100,000IU	100,000IU	100,000IU	100,000IU
Phenylalanine	1 mg	1 mg	1 mg	1 mg	1 mg	-
Arginine hydrochloride	0.2 mg	0.5 mg	1.2 mg	1.5 mg	-	-
Pullulan	-	-	-	-	-	2 mg
Disintegration Index	0.269	0.251	0.235	0.247	0.232	0.001
Fine particle fraction	59%	55%	48%	50%	77%	0%
Residual activity after freeze-drying	70%	77%	100%	98%	56%	- #1
Residual activity after high-temperature preservation	100%	100%	100%	97%	21%	- #1

Note: #1 The residual activity after freeze-drying and residual activity after high-temperature preservation for the comparative example 2 were not measured.

[0052]

The freeze-dried compositions obtained in Examples 1 to 4 and the Comparative Example 1 were in the form of a non-powder cake-like lump (freeze-dried cake) after freeze-drying. As shown in Table 1, the freeze-dried compositions obtained in Examples 1 to 4 and Comparative Example 1 were easily made into fine particles in the vessel by an air impact arising through an air speed of about 35 m/sec and an air flow rate of about 40 ml/sec, and thus obtained a suitable fine particle fraction. Therefore, it was verified that the freeze-dried compositions obtained in Examples 1 to 4 and Comparative Example 1 were possible to produce a powdered preparation suitable for transpulmonary administration. The freeze-dried composition containing pullulan as a carrier was not disintegrated by the air impact, and did not form fine particles.

[0053]

Moreover, it was verified that the freeze-dried compositions obtained in Examples 1 to 4 maintained a high IFN-γ activity due to the freeze-drying process as compared to the freeze-dried composition containing no hydrophilic amino acids in Comparative Example 1. It was also verified that IFN-γ in the freeze-dried composition containing no hydrophilic amino acids in Comparative Example 1

deactivated under an extremely severe temperature conditions (70°C) while the freeze-dried compositions containing hydrophobic amino acids and hydrophilic amino acids obtained in Examples 1 to 4 maintained high IFN-γ activity under such temperature conditions.

[0054]

Examples 5 to 11

An interferon-γ (IFN-γ) stock liquid (potency: 1×10^7 IU/ml) was desalinated using an ultrafilter membrane (Ultrafree 15, manufactured by Millipore). 100,000 IU or 1,000,000 IU of the desalinated IFN-γ obtained and various carriers having the amount shown in Table 2 were dissolved into distilled water for an injection such that the total amount was 0.5 ml and the resultant was filled into vessels (trunk diameter 18 mm), and freeze-drying was carried out using a shelf-type freeze-dryer (Lyovac GT-4, made by Leybold) (Examples 5 to 11).

[0055]

The disintegration index of the non-powder-form freeze-dried composition (freeze-dried cake) obtained was calculated. The disintegration index of the non-powder-form freeze-dried composition (freeze-dried cake) obtained was calculated.

[0056]

Next, a vessel filled with the non-powder-form

freeze-dried composition (freeze-dried cake) obtained in Examples 5 to 11 was installed in a jet type dry powder inhaler (having a bellows body 10 capable of supplying an amount of air of about 20ml; Fig. 1) designed such that the bore of the air jet flow path 3 was 1.2 mm and the bore of the discharge flow path 4 was 1.8 mm. This inhaler was attached to an Aerosizer (made by Amherst Process Instrument, Inc., USA) fitted with an Aerobreather, which is an artificial lung model, and an amount of air of about 20 ml was introduced into the vessel from the inhaler, thus applying an air impact arising through an air speed of about 35 m/sec and an air flow rate of about 40 ml/sec to the freeze-dried cake. As a result, air was introduced from the air jet flow path 3 of the jet type dry powder inhaler into the vessel 1, and it was observed that the non-powder-form freeze-dried composition in the vessel was made into fine particles by the air impact. The particle size distribution of the fine particles was measured using the Aerosizer fitted with the Aerobreather (measurement conditions: breath rate: 60 L/min, breath volume: 1 L, acceleration: 19). The mass median aerodynamic diameter (μm ± SD) was then calculated from the particle size distribution of the fine particles jetted out from the inhaler.

[0057]

The fine particle fraction (%), residual activity after freeze-drying (%) and residual activity (%) after high-temperature preservation for each freeze-dried composition were evaluated in the same manner as in Examples 1 to 4.

[0058]

The freeze-dried compositions obtained in Examples 5 to 11 were in the form of a non-powder cake-like lump (freeze-dried cake) after freeze-drying. As shown in Table 2, the freeze-dried compositions obtained in Examples 5 to 11, which showed a disintegration index of at least 0.15, were easily made into fine particles in the vessel by an air impact arising through an air speed of abcut 35 m/sec and an air flow rate of about 40 ml/sec, and thus obtained a fine particle fraction having a mass median aerodynamic diameter of 5 microns or less, and hence it was possible to produce preparations suitable for transpulmonary administration. Each freeze-dried composition showed a favorable fine particle fraction. Moreover, it was verified that the freeze-dried composition obtained in Examples 5 to 11 showed high residual activity after freeze-drying and residual activity after high-temperature preservation, and also maintained high IFN-γ activity even in the preparation of a composition and under conditions of high-temperature

preservation.

[Table 2]

	Ex. 5	Ex. 6	Ex. 7	Ex. 8	Ex. 9	Com. Ex. 10	Com. Ex. 11
IFN-γ (IU)	100,000	100,000	100,000	1,000,000	1,000,000	1,000,000	1,000,000
Phenylalanine	1.2 mg	1.2 mg	1.2 mg	1 mg	1 mg	1 mg	-
Leucine	0.3 mg	-	-	0.3 mg	-	-	-
Valine	-	0.3 mg	-	-	0.3 mg	-	0.8 mg
Isoleucine	-	-	0.3 mg	-	-	0.3 mg	-
Arginine hydrochloride	0.2 mg	0.2 mg	0.2 mg	0.2 mg	0.2 mg	0.2 mg	0.2 mg
Disintegration Index	0.191	0.190	0.181	0.316	0.293	0.281	0.150
Mass median aerodynamic diameter (μm ± SD, MMDA)	1.537 ± 1.438	1.698 ± 0.542	1.874 ± 1.842	1.278 ± 0.386	1.387 ± 1.591	1.964 ± 1.673	1.597 ± 1.625
Fine particle fraction	67%	64%	67%	85%	82%	78%	70%
Residual activity after freeze-drying	83%	80%	84%	100%	92%	97%	80%
Residual activity after high-temperature preservation	93%	95%	98%	93%	98%	78%	87%

[0060]

Reference Example 1

The following tests were conducted to determine the effects of salts contained in the freeze-dried composition on powderizeation by air impact.

[0061]

Interferon-α (IFN-α), various amino acids and citrates (citric acid and sodium citrate) having the amounts shown in Table 3 were dissolved into distilled water for an injection such that the total amount was 0.5 ml. The resultant was filled into vessels (trunk diameter 18 mm), and freeze-drying was carried out using a shelf-type freeze-dryer (Lyovac GT-4, made by Leybold) (Examples 1 to 5). The disintegration index and fine particle fraction of the non-powder-form freeze-dried compositions (freeze-dried cake) obtained were calculated in the same manner as in Examples 1 to 4.

[0062]

The obtained results were shown in Table 3. As can be seen from Table 3, it was verified that the disintegration index was increased when the content of citrate in the freeze-dried composition was small. Moreover, it was verified that the proportion of effective particles was excellent when the content of citrate in the freeze-dried composition was small.

[0063]

[Table 3]

	Ref. Ex. 1	Ref. Ex. 2	Ref. Ex. 3	Ref. Ex. 4	Ref. Ex. 5
IFN-α	10,000,000IU	10,000,000IU	10,000,000IU	10,000,000IU	10,000,000IU
Leucine	1.8 mg	1.8 mg	1.8 mg	1.8 mg	1.8 mg
Valine	1.2 mg	1.2 mg	1.2 mg	1.2 mg	1.2 mg
Citrate	-	0.06 mg	0.12 mg	0.24 mg	0.49 mg
Disintegration Index	0.237	0.245	0.218	0.207	0.198
Fine particle fraction	74%	66%	65%	63%	53%

[0064]

Reference Example 2

The following tests were conducted to determine the effects of salts contained in the freeze-dried composition on powderization by air impact.

[0065]

Interferon-α (IFN-α), various amino acids and phosphates (sodium dihydrogenphosphate dihydrate and disodium hydrogenphosphate dodecahydrate) having the amounts shown in Table 4 were dissolved into distilled water for an injection such that the total amount was 0.5 ml. The resultant was filled into vessels (trunk diameter 18 mm), and freeze-drying was carried out using a shelf-type freeze-dryer (Lyovac GT-4, made by Leybold) (Examples 6 to 8). The disintegration index and fine particle fraction of the non-powder-form freeze-dried

compositions (freeze-dried cake) obtained were calculated in the same manner as in Examples 1 to 4.

[0066]

The obtained results were shown in Table 4. As can be seen from Table 4, it was verified that the disintegration index was increased and the proportion of effective particles became higher when the content of phosphate in the freeze-dried composition was low.

[0067]

[Table 4]

	Ref. Ex. 6	Ref. Ex. 7	Ref. Ex. 8
IFN-α	1,000,000IU	1,000,000IU	1,000,000IU
Leucine	1.5 mg	1.5 mg	1.5 mg
Valine	1 mg	1 mg	1 mg
Phosphate	-	0.05 mg	0.5 mg
Disintegration Index	0.185	0.196	0.168
Fine particle fraction	59%	55%	44%

[0068]

The results obtained from Reference Examples 1 and 2 show that salts contained in the non-powder-form freeze-dried composition inhibit the composition from being made into fine particles. Thus, it was verified that the disintegration index

was increased and the proportion of effective particles became higher when the content of salts contained in the non-powder-form freeze-dried composition was low. More specifically, the non-powder-form freeze-dried composition which can be prepared by an air impact into fine particles having an excellent proportion of effective particles can be obtained by reducing the concentration of the salts contained in the solution used for freeze-drying.

[0069]

[Effects of the Invention]

The freeze-dried composition for transpulmonary administration of the present invention can be made into fine particles down to the size necessary for delivery into the lungs by an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec. Therefore, a user (patient) himself/herself can prepare the freeze-dried composition into a powdered preparation comprising fine particles suitable for transpulmonary administration at the time of use (in particular, the time of inhalation) using a simple means.

[0070]

The proportion of effective particles (fine particle fraction) attained by the freeze-dried composition for transpulmonary administration of the present invention is at least 10%, and can be increased to at least 20%, at least 25%, at least 30% or at least 35%. U.S. Patent No. 6153224 indicates

that, with many of prior art dry powder inhalers, the proportion of the active ingredient (particles) to adhere to the lower portions of the lungs is only about 10% of the amount of the active ingredient inhaled. Further, Japanese Unexamined Patent Publication No. 2001-151673 states that the amount of an inhalation powder preparation reaching the lungs (lung reaching proportion) is generally about 10% of the drug discharged from the preparation. Therefore, the freeze-dried interferon-γ composition for transpulmonary administration of the present invention is valuable in that it is capable of achieving a higher proportion of effective particles (fine particle fraction) than prior art powder inhalation preparations.

[0071]

The freeze-dried composition for transpulmonary administration of the present invention has a cake-like form, which eliminates the need for subdividing into vessels the fine particle powders which are difficult to handle. Therefore, the freeze-dried composition for transpulmonary administration of the present invention can be prepared at high preparatory yield as compared to fine particle powder-form compositions for transpulmonary administration, and moreover, can avoid contamination with impurities due to subdividing the fine particle powder form into vessels.

[0072]

Moreover, the freeze-dried interferon-γ composition for

transpulmonary administration of the present invention can maintain IFN-γ stably. Therefore, the activity of IFN-γ can be maintained at high ratios even when subjected to a freeze-drying process during preparation or a long-period of preservation.

[Document Name] Abstract

[Summary]

[Object]

It is an object of the present invention to provide a composition containing interferon-γ for transpulmonary administration which can stably maintain interferon-γ and can be made into fine particles in the vessel at the time of usage.

[Method for Achieving the Object]

The present invention provides a freeze-dried composition for transpulmonary administration having the following properties (i) to (iv):

(i) containing hydrophobic stabilizer, hydrophilic stabilizer and interferon-γ;

(ii) a non-powder cake-like form;

(iii) a disintegration index of 0.015 or more; and

(iv) becoming fine particles having a mean particle diameter of 10 microns or less or a fine particle fraction of 10% or more upon receipt of an air impact having an air speed of at least 1 m/sec and an air flow rate of at least 17 ml/sec.

[Selected Figure] None